

Appl. No.: 09/823,373  
DRAFT Reply and Amendment for discussion purposes only  
To Final Office Action, mailed 22 April

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#### IN THE CLAIMS:

1. (Cancelled)

2. (Currently Amended) An isolated nucleic acid fragment comprising a first nucleotide sequence encoding a polypeptide with nitrilase activity having at least ~~90~~ 95 % identity as compared to a polypeptide encoded by the sequence identified in SEQ ID NO:5, or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

3.-8. (Cancelled)

9. (Currently amended) A chimeric gene comprising the isolated nucleic acid fragment of ~~Claims 1 or Claim 2~~ operably linked to suitable regulatory sequences.

10. (Previously presented) A plasmid pnitex2 contained in *E. coli* SS1001 having the designation ATCC PTA-1177.

11. (Original) An expression cassette comprising the chimeric gene of Claim 9.

12. (Previously presented) The expression cassette of Claim 11 comprising plasmid pnitex2.

13. (Original) A transformed microorganism comprising the chimeric gene Claim 9.

14. (Original) A transformed microorganism comprising the plasmid of Claim 10.

15. (Original) A transformed microorganism comprising the expression cassette of Claim 11.

16. (Original) The transformed microorganism of Claim 15 wherein the expression cassette is chromosomally integrated.

17. (Original) The transformed microorganism of Claim 16 further comprising suitable regulatory sequences.

18. (Original) The transformed microorganism of Claim 17 wherein the suitable regulatory sequences comprise

- a) at least one promoter selected from the group consisting of the tryptophan operon promoter P<sub>trp</sub> of *E. coli*, a lactose operon promoter Plac of *E. coli*, a P<sub>tac</sub> promoter of *E. coli*, a phage lambda right promoter P<sub>R</sub>, a phage lambda left promoter P<sub>L</sub>, a T7 promoter, a promoter of the AOX1 gene from *Pichia pastoris*, and a promoter of the GAP gene from *Pichia pastoris*, or is at least one strong promoter selected from the group consisting of *Comamonas*, *Corynebacterium*, *Brevibacterium*, *Rhodococcus*, *Azotobacter*, *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Salmonella*, *Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Pichia*, *Zygosaccharomyces*, *Kluyveromyces*, *Candida*, *Hansenula*, *Dunaliella*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacteria*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Rhizobium*, and *Streptomyces*, and
- b) at least one ribosome binding site from a phage lambda CII gene or selected from the group consisting of ribosome binding sites from a gene of *Comamonas*,

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*Corynebacterium, Brevibacterium, Rhodococcus, Azotobacter, Citrobacter, Enterobacter, Clostridium, Klebsiella, Salmonella, Lactobacillus, Aspergillus, Saccharomyces, Zygosaccharomyces, Pichia, Kluyveromyces, Candida, Hansenula, Dunaliella, Debaryomyces, Mucor, Torulopsis, Methylobacteria, Bacillus, Escherichia, Pseudomonas, Rhizobium, and Streptomyces.*

19. **(Original)** The transformed microorganism of Claim 18, wherein the host microorganism is selected from the group consisting of *Comamonas, Corynebacterium, Brevibacterium, Rhodococcus, Azotobacter, Citrobacter, Enterobacter, Clostridium, Klebsiella, Salmonella, Lactobacillus, Aspergillus, Saccharomyces, Zygosaccharomyces, Pichia, Kluyveromyces, Candida, Hansenula, Dunaliella, Debaryomyces, Mucor, Torulopsis, Methylobacteria, Bacillus, Escherichia, Pseudomonas, Rhizobium, and Streptomyces.*

20. **(Previously presented)** A transformed microorganism *E. coli* SS1001 having the designation ATCC PTA-1177.

21.-45 **(Cancelled)**

46. **(Original)** The transformed microorganism of Claim 19, wherein the host microorganism is *E. coli* strains MG1655 (ATCC 47076), W3110 (ATCC 27325), MC4100 (ATCC 35695), or W1485 (ATCC 12435).

47. **(Previously presented)** An isolated nucleic acid fragment encoding a polypeptide having the amino acid sequence of SEQ ID NO:5.

48. **(Cancelled)**

49. **(Previously presented)** An isolated nucleic acid fragment comprising a first nucleotide sequence encoding a polypeptide with nitrilase activity having at least 95 % identity as compared to a polypeptide encoded by the sequence identified in SEQ ID NO:5.